APPLIED AND ENVIRONMENTAL MICROBIOLOGY, May 1990, p. 1476–1479
0099-2240/90/051476-04$02.00/0

Vol. 56, No. 5

Type of Closure Prevents Microbial Contamination of Cosmetics during Consumer Use

DANIEL K. BRANNAN[1]* AND JAMES C. DILLE[2]

Department of Biology, Abilene Christian University, Abilene, Texas 79699,[1] and Beauty Care Division, The Procter & Gamble Co., Cincinnati, Ohio 45241[2]

Received 18 December 1989/Accepted 21 February 1990

The dispensing closure used for containers plays an important role in protecting cosmetics from in-use microbial contamination. This hypothesis was tested by aseptically packing unpreserved shampoo and skin lotion into containers with three different closure types which provided various degrees of protection against consumer and environmental microbial insults. Shampoo was packed in containers with slit-cap (n = 25), flip-cap (n = 25), or screw-cap (n = 28) closures. Skin lotion was packed in containers with pump-top (n = 21), flip-cap (n = 18), or screw-cap (n = 21) closures. The products were then used by volunteers under actual in-use conditions for 3 (shampoo) or 2 (skin lotion) weeks. After use, the products were evaluated for microbial contamination by using standard methods for enumeration and identification. The standard screw-cap closure provided only minimal protection against microbial contamination of both the shampoo (29% contamination incidence) and the skin lotion (71%). The slit-cap closure on the shampoo container and the flip-cap closure on the skin lotion container provided slightly enhanced degrees of protection (21 and 39% contamination incidence, respectively). The greatest amount of protection (i.e., lowest contamination incidence) was provided by the flip-cap closure for the shampoo container (0%) and the pump-top closure for the skin lotion container (10%). As a result, closure type plays an important role in protecting poorly preserved products from in-use microbial contamination.

Our hypothesis in the work described here is that the dispensing closure used for containers plays an important role in preventing contamination of cosmetics during use. Therefore, even poorly preserved products may withstand consumer use without becoming contaminated if packaged with closures that provide adequate protection from consumer and environmental microbial insult. To our knowledge, the work described in this report is the first to determine the positive role that container closures have in protecting cosmetics from in-use contamination.

The few attempts to correlate microbial challenge tests with consumer contamination potential have been inadequate to prevent the Food and Drug Administration from issuing a Federal Register notice stating that regulatory action would be taken "to remove from the market any cosmetic that poses an unreasonable risk of injury because of inadequate preservation to withstand contamination under customary conditions of use" (12). None of the studies conducted before or since this 1977 notice has been adequate for the establishment of guidelines or regulations by the Food and Drug Administration for preservative testing protocols (1–3, 6, 10, 11, 14, 19, 22). Currently, the Association of Official Analytical Chemists is proposing to establish standard testing protocols for cosmetics which the Food and Drug Administration may adopt.

We previously described a microbial challenge test that accurately predicted the risk of consumer contamination of cosmetics during ordinary use (8). The test was able to categorize two classes of cosmetics (shampoos and skin lotions) as either poorly preserved, marginally preserved, or well preserved. These test results correlated well with consumer in-use results. Products classified by the test as poorly preserved were heavily contaminated during use; products classified as marginally preserved had a low contamination level; and products classified by the test as well preserved did not become contaminated after consumer use.

In our original study (8), we showed that the challenge test we developed accurately predicted the susceptibility of shampoos and skin lotions to microbial contamination from consumer use. The test we described, however, assessed only the ability of the preservative system of the product to prevent in-use contamination. The role that packaging played in protecting a product was not determined. Container design, specifically the dispensing closure, may also play an important role in protecting a product from consumer or environmental contamination during use. For example, caps that provide minimal exposure of a product to consumer or environmental contact would be expected to provide greater protection from contamination than those that do not minimize exposure. This paper provides evidence that dispensing closure plays an important role in preventing contamination.

MATERIALS AND METHODS

Packaging evaluated. Two product types (shampoo and skin lotion) were evaluated in packages with three levels of protection from in-use contamination. The shampoo was packaged in standard 16-oz (ca. 473 ml) bottles with three different closures as follows: a standard screw-cap, which provided a large (24 mm in diameter) opening for the product; a flip-cap, which provided a small (6 mm) opening for the product (which was protected by a hinged cap); and a slit-cap, which provided no direct opening for the product (which was protected by a hinged cap) (Fig. 1).

The skin lotion was packaged in 4- to 10-oz (ca. 118- to 296-ml) bottles equipped with three different closures as follows: a standard screw-cap, which provided a large (15 mm in diameter) opening into the product; a flip-cap, which provided a small (6 mm) opening protected by a hinged cap; and a pump-top, which provided no direct opening (Fig. 1).

* Corresponding author.

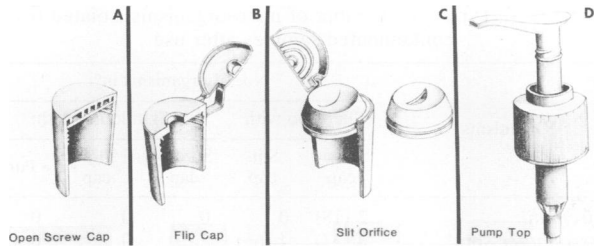

FIG. 1. Closure types used to evaluate the degree of protection afforded unpreserved shampoo and skin lotion from consumer contamination during use. (A) Open screw-cap used for shampoo and skin lotion; (B) flip-cap used for shampoo and skin lotion; (C) slit-cap used for shampoo; (D) pump-top used for skin lotion.

Description of products. Both products were made without preservatives and thus were packed under aseptic conditions. Because of the absence of preservatives, the formulae had little or no inherent hostility to microbes. This lack of hostility was confirmed by the methods described below. The shampoo was composed (in descending order of ingredient concentration) of water, ammonium lauryl sulfate, sodium lauryl sulfate, cocamide diethanolamide, polyquaternium 10, sodium phosphate, fragrance, SD alcohol 40, sodium chloride, disodium phosphate, and color.

The skin lotion was composed (in descending order of ingredient concentration) of water, glycerol, petrolatum, cetyl alcohol, cyclomethicone and dimethicone copolyol, stearyl alcohol, isopropyl palmitate, dimethicone, sodium hydroxide, stearic acid, lanolin acid, polyethylene glycol 100 stearate, carbomer 934, EDTA, hydrogenated vegetable glycerides, phosphate, masking fragrance, and titanium dioxide.

Microbial challenge test. Our previously validated challenge test was used to confirm lack of product hostility; the details of this test (e.g., organisms and concentrations used) have been described previously (8). The test involves inoculating various dilutions of the product with a variety of preservative-resistant microorganisms and following the course of their elimination from the product over 28 days. The test is a modification of the standard procedure of the Cosmetics, Toiletries, and Fragrance Association, Inc. (9).

Consumer use test. Approximately 20 subjects were randomly assigned to each closure and product type; they were asked to use the products as they normally would. All products were provided to the subjects free of detectable microorganisms (<20 CFU/g). Unexposed control products for each cap and product type were incubated during the test period to ensure that no lapses in aseptic packing occurred. These controls all remained below the detectable limit throughout the test. Skin lotion products were returned after 2 weeks of consumer use. The shampoos were returned after 3 weeks of use.

Microbial content testing was conducted on each returned product immediately upon receipt and 4 to 7 days postreceipt. Previously described standard techniques for microbial content testing were used (8). A product was considered contaminated if >100 CFU/g of product was observed or if gram-negative bacteria at any level were detected both upon initial receipt and 4 to 7 days postreceipt.

Bacteria were identified by using the API 20E, API NFT, and API Staph-trac (Analytab Products); Enterotube (Hoffmann-La Roche Inc.); or Oxi-ferm (Hoffmann-La Roche) rapid identification system.

Statistical analyses. Chi-square, analysis of variance, and the Shannon diversity index tests were performed on the data (23).

RESULTS

Microbial challenge testing. Both the shampoo and the skin lotion failed the challenge test, indicating that both products were poorly preserved and thus susceptible to consumer contamination. For comparison, these results and the results of well-preserved control products are provided in Table 1.

TABLE 1. Microbial challenge of unpreserved shampoo and skin lotion used for evaluating closure ability to resist in-use consumer contamination

Product[a]	Product concn (%)	CFU/g of product at day postchallenge					
		0	1	7	14	21	28
Unpreserved shampoo in all cap types	100	TNTC[b]	TNTC	TNTC	TNTC	TNTC	TNTC
	70	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
	50	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
	30	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
Unpreserved skin lotion in all cap types	100	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
	70	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
	50	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
	30	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC
Well-preserved shampoo	100	TNTC	260	<20	<20	<20	<20
	70	TNTC	1,200	<20	<20	<20	<20
	50	TNTC	2,020	<20	<20	<20	<20
	30	TNTC	11,000	<20	<20	<20	<20
Well-preserved skin lotion	100	TNTC	80	<20	<20	<20	<20
	70	TNTC	TNTC	<20	<20	<20	<20
	50	TNTC	TNTC	<20	<20	<20	<20
	30	TNTC	TNTC	40	20	<20	<20

[a] Preserved formulae identical to unpreserved except for the addition of preservatives. Methyl- and chloroisothiazolinones were added to the shampoo; imidazolidinyl urea and methyl- and propylparabens were added to the skin lotion.

[b] TNTC, Too numerous to count.

TABLE 2. Unpreserved shampoo and skin lotion protection with three closure types from in-use consumer contamination[a]

Cosmetic and closure	No. of subjects	Avg amt used (g)	Avg no. of uses	% Samples contaminated
Shampoo				
Screw-cap	28	96]	17]	29 (8/28)]
Slit-cap	25	131]	14]	21 (6/25)]
Flip-cap	25	98]	14]	0 (0/25)]
Skin lotion				
Screw-cap	21	32]	18]	71 (14/21)]
Flip-cap	18	36]	23]	39 (7/18)]
Pump	21	35]	22]	10 (2/21)]

[a] Bracketed values are not significantly different ($\alpha = 0.05$).

Poorly preserved products were used in this study to allow an assessment of the role that closure design alone has in protecting products from contamination in the absence of preservative protection.

In-use testing. In-use microbial contamination of the shampoo and skin lotion for each closure type is shown in Table 2. Usage data are also shown. The total number of shampoo uses as well as the actual amounts of product used from the shampoo containers with the three closure types were statistically equivalent. The difference in contamination with the standard screw-cap or slit-cap was not statistically significant. However, shampoo with the standard screw-cap showed a directionally higher contamination incidence (29%) than did the slit-cap (21%). The closure offering the greatest degree of protection from contamination was the flip-cap (0% contamination).

Total skin lotion uses and total amounts used were not significantly different. Contamination with the standard screw-cap was highest (71%) and statistically different from contamination with the flip-cap (39%) and the pump-top (10%). The pump-top dispenser afforded the greatest protection but did not completely eliminate contamination potential.

Types and levels of microorganisms in products. Table 3 shows the type and incidence of microorganisms found in each product with each type of cap after use. The isolates found most frequently in the contaminated shampoo were *Enterobacter* spp., *Serratia* spp., and *Citrobacter freundii*; *Klebsiella* spp. and *Pseudomonas* spp. were also isolated. The organisms most frequently encountered in the contaminated skin lotion were yeasts and molds, followed by *Pseudomonas* spp., *Klebsiella* spp., and *Enterobacter* spp.

Table 4 presents the contamination incidence, diversity index, and contamination levels for the products. A measure of which cap types permitted the greatest variation in types of microbial contaminants was made by using the Shannon diversity index (23). Maximum diversity as measured by the Shannon index is $J' = 1.0$. The screw-cap for both the shampoo and skin lotion permitted the greatest diversity, while the slit-cap for the shampoo and flip-cap and pump-top for the skin lotion permitted less variation in types of contaminants.

The average contamination level (log normalized) of the shampoo was higher with the screw-cap than with the slit-cap (Table 4), whereas the average contamination level of the skin lotion with the screw-cap was higher than that with the flip-cap. The pump-top used on the skin lotion, despite the fact that it permitted only 10% of the units to become contaminated, permitted the highest overall contamination levels.

TABLE 3. Types and number of microorganisms isolated from contaminated samples after use

Organisms	No. of organisms in[a]:				
	Shampoo with:		Skin lotion with:		
	Screw-cap	Slit-cap	Screw-cap	Flip-cap	Pump
C. freundii	2 (18)	0	0	0	0
Enterobacter spp.[b]	4 (37)	4 (66)	2 (9)	0	0
Klebsiella spp.[c]	1 (9)	1 (17)	2 (9)	0	1 (33)
Pseudomonas spp.[d]	1 (9)	1 (17)	5 (21)	1 (12.5)	1 (33)
Serratia spp.[e]	2 (18)	0	1 (4)	0	0
GNR[f] (nonfermentative)	0	0	1 (4)	1 (12.5)	0
GNR (fermentative)	1 (9)	0	0	0	0
CDC serotype IVC2	0	0	1 (4)	0	0
Bacillus sp.	0	0	1 (4)	0	0
Staphylococcus epidermidis	0	0	1 (4)	0	1 (33)
Propionibacterium sp.	0	0	1 (4)	0	0
Sarcina sp.	0	0	1 (4)	0	0
Diphtheroid	0	0	1 (4)	0	0
Yeasts and molds	0	0	7 (29)	6 (75)	0

[a] Values in parentheses are the percentages of each isolate in the total number of isolates recovered from that particular product. In some cases, several isolates were recovered from a single product and cap.
[b] *E. aerogenes*, *E. agglomerans*, and *E. cloacae*.
[c] *K. pneumoniae* and *K. oxytoca*.
[d] *P. putida*, *P. fluorescens*, *P. paucimobilis*, *P. aeruginosa*, and *P. maltophilia*.
[e] *S. liquefaciens*, *S. odorifera*, and *S. rubidaea*.
[f] GNR, Gram-negative rod.

In our study, we found that flip-caps protect even poorly preserved shampoos from consumer contamination. The overall incidence of contamination, diversity of contaminants, and actual levels of contamination were all lower than those with any of the other closures used for the shampoo. The slit-cap protected shampoos better than the screw-cap, as seen by a directionally lower degree of contamination incidence, lower diversity index, and lower levels of contamination than with the screw-cap.

The pump-top closure provided the lowest incidence of contamination for the skin lotion, but there was a slightly directionally higher diversity index and higher overall contamination level than with the flip-cap. The skin lotion with the screw-cap had the highest contamination incidence, highest diversity index, and a higher level of contamination than the lotion with the flip-cap closure.

TABLE 4. Contamination incidence, average contamination level, and diversity of microbial contaminants in unpreserved shampoo and skin lotion with various closures

Product and closure	% In-use contamination[a]	Log-normalized avg contamination (CFU/g of product)	Diversity of contaminants[b]
Shampoo			
Screw-cap	29]	2.37×10^5	0.92
Slit-cap	21]	1.97×10^3	0.49
Flip-cap	0]	0	0
Skin lotion			
Screw-cap	71]	2.61×10^4	0.87
Flip-cap	39]	5.62×10^3	0.30
Pump	10]	9.49×10^5	0.44

[a] Bracketed values are not significantly different ($\alpha = 0.05$).
[b] Shannon diversity index (J') of 1.0 indicates a high diversity of contaminating genera. Values less than 0.5 indicate little diversity.

DISCUSSION

Closure design plays an important yet heretofore unrecognized role in protecting cosmetics from consumer contamination. On the basis of results reported here, even poorly preserved products can receive some degree of protection from consumer use if packaged correctly. Manufacturing concerns, such as raw materials with bioloads and the need to prevent microbial adaptation to products, usually dictate that preservatives be added to protect products from contamination during the making. Product protection due to this preservation is typically sufficient to prevent consumer contamination. Preservative protection coupled with good closure design, however, provides an even higher degree of protection for the consumer from contamination of the product during use.

Both the flip-cap and the slit-cap closures provided better protection of poorly preserved shampoos than the screw-cap closure. This result is likely due to the fact that the screw-cap closure permits relatively high exposure to environmental and consumer contamination. Similarly, both the pump-top and the flip-cap closures provided a better degree of protection for poorly preserved skin lotions against consumer contamination than screw-cap closures. The types of organisms recovered from the contaminated products were those found indigenous to humans and household environments (13, 17, 20). Therefore, the microbial contaminants found in the contaminated products were reflective of the environments to which they were exposed.

Inadequately protected cosmetics, whether because of poor preservation or closure designs that permit access to the environment, may become contaminated with undesirable organisms during use (1–3, 11, 22). Contamination leads to product degradation or, if it is contaminated with pathogens, allows the product to act as a fomite to potentially spread infection to susceptible users (4, 15, 16, 18, 21). Microbial contamination of cosmetic products may also occur during their manufacture (5, 7). While microbial contamination from manufacturing is controlled by careful attention to sanitary processing and adequate preservation, contamination from consumer use is controlled by product preservation and, as our results show, by container designs that provide for minimal exposure of the product to consumer and environmental microbial insult.

ACKNOWLEDGMENTS

This work was supported by the Procter & Gamble Co. while D. K. Brannan was in their employ.

LITERATURE CITED

1. **Ahearn, D. G., J. Sanghvi, G. J. Haller, and L. A. Wilson.** 1978. Mascara contamination: in use laboratory studies. J. Soc. Cosmet. Chem. **29**:127–131.
2. **Ahearn, D. G., L. A. Wilson, A. J. Julian, D. J. Reinhardt, and G. Ajello.** 1974. Microbial growth in eye cosmetics: contamination during use. Dev. Ind. Microbiol. **15**:211–215.
3. **Anderson, D. W., and M. Ayers.** 1972. Microbiological profile of selected cosmetic products with and without preservatives after use. J. Soc. Cosmet. Chem. **23**:863–873.
4. **Anderson, K.** 1962. The contamination of hexachlorophene soap with *Pseudomonas pyocyanea*. Med. J. Aust. **49**:463–465.
5. **Baird, R. M.** 1984. Bacteriological contamination of products used for skin care in babies. Int. J. Cosmet. Sci. **6**:85–90.
6. **Bhadauria, R., and D. G. Ahearn.** 1980. Loss of effectiveness of preservative systems of mascaras with age. Appl. Environ. Microbiol. **39**:665–667.
7. **Bowman, F. W., E. W. Knoll, M. White, and P. Mislivec.** 1972. Survey of microbial contamination of ophthalmic ointments. J. Pharm. Sci. **61**:532–535.
8. **Brannan, D. K., J. C. Dille, and D. J. Kaufman.** 1987. Correlation of in vitro challenge testing with consumer use testing for cosmetic products. Appl. Environ. Microbiol. **53**:1827–1832.
9. **Cosmetics, Toiletries, and Fragrance Association, Inc.** 1983. Determination of adequacy of preservation of cosmetic and toiletry formulations. CTFA technical guideline. Cosmetics, Toiletries, and Fragrance Association, Inc., Washington, D.C.
10. **Cowen, R. A.** 1974. Relative merits of 'in use' and laboratory methods for the evaluation of antimicrobial products. J. Soc. Cosmet. Chem. **25**:307–323.
11. **Dawson, N. L., and D. J. Reinhardt.** 1981. Microbial flora of in-use, display eye shadow testers and bacterial challenges of unused eye shadows. Appl. Environ. Microbiol. **42**:297–302.
12. **Federal Register.** 1977. Notice of intent to propose regulations and request for information on preservation of cosmetics coming into contact with the eye. Fed. Regist. **45**:54837–54838.
13. **Finch, J. E., and M. Hanksworth.** 1978. A bacteriological survey of the domestic environment. J. Appl. Bacteriol. **45**:357–364.
14. **Lindstrom, S. M.** 1986. Consumer use testing: assurance of microbial product safety. Cosmet. Toiletries **101**:71–73.
15. **Morse, L. J., and L. E. Schonbeck.** 1968. Hand lotions—potential nosocomial hazard. N. Engl. J. Med. **278**:376–378.
16. **Morse, L. J., H. L. Williams, P. F. Green, E. E. Eldridge, and J. R. Rotta.** 1967. Septicemia due to *Klebsiella pneumoniae* originating from a hand cream dispenser. N. Engl. J. Med. **277**:472–473.
17. **Noble, W. C.** 1981. Microbiology of the human skin, 2nd ed. Lloyd Luke, London.
18. **Noble, W. C., and J. A. Savin.** 1966. Steroid cream contamination with *Pseudomonas aeruginosa*. Lancet **i**:347–349.
19. **Rdzok, E. J., W. E. Grundy, F. J. Kirchmeyer, and J. C. Sylvester.** 1955. Determining the efficacy of preservatives in cosmetic products. J. Am. Pharm. Assoc. **44**:613–616.
20. **Scott, E., S. F. Bloomfield, and C. G. Barlow.** 1982. An investigation of microbial contamination in the home. J. Hyg. **89**: 279–293.
21. **Wilson, L. A., and D. G. Ahearn.** 1977. *Pseudomonas*-induced corneal ulcers associated with contaminated eye mascaras. Am. J. Ophthalmol. **84**:112–119.
22. **Wilson, L. A., A. J. Julian, and D. G. Ahearn.** 1975. The survival and growth of microorganisms in mascara during use. Am. J. Ophthalmol. **29**:596–601.
23. **Zar, J. H.** 1984. Biostatistical analysis, 2nd ed. Prentice-Hall, Inc., Englewood Cliffs, N.J.